elastic stiffness of these two regions, the experiments have revealed the presence of two types of membrane domains, with different mechanical and adhesive properties, both distributed all over the oocyte surface, i.e. in both microvillar and amicrovillar regions. If the gamete contact occurs on the first type of domains, the oocyte membrane deforms only elastically under traction. The pulloff forces in these domains are higher in the amicrovillar region. For a contact of the spermatozoon on the other type of domains, there can be a transition from elastic to viscoelastic regime and then tethers are extruded from the oocyte membrane.

1831-Plat Nonlinear Response Probed by Laser-Trapping Microrheology

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Microrheology can be used to probe the viscoelastic properties of materials on micrometer scales and is thus applicable in cells. Cytoskeletal polymers are in general semiflexible and have strong non-linear elastic responses. Nonlinear response can not be probed by "passive" microrheology which relies on thermal fluctuations. We have here developed a high-bandwidth technique for active 2-particle microrheology with which we can probe linear and nonlinear responses of soft materials. Micron-sized colloidal probe particles are driven by an oscillating optical trap, and the resulting correlated motions of neighboring particles are detected by laser interferometry. Lock-in detection at the driving frequency and at its second harmonic makes it possible to measure the linear and the non-linear response of the embedding medium at the same time. We demonstrate the sensitivity of the method by detecting a second-harmonic response in water which is of purely geometric origin and which can be fully understood within linear hydrodynamics.

1832-Plat Monitoring the Drug-Induced Rheological Response of a Live Cell through Bio-Microrheology

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Bio-microrheology, the study of deformation and flow of biological material at the microscale, is applicable to a range of biomaterials from biopolymers to live cells. We use bio-microrheology techniques, primarily live-cell particle tracking, to monitor the time-dependent rheological response of various adherent cell lines to specific drugs. In addition to developing appropriate experimental protocols and data analysis methods, we seek a deeper understanding of the link between biochemical drug pathways and cell rheology.

Symposium 17: From Protein Crystals to Amyloid Fibrils: Condensed Colloidal Phases in Biology (APS/BPS)

1833-Symp Phase Transitions in Protein Solutions

Aleksey Lomakin

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Globular proteins in aqueous solutions show transitions between dilute, concentrated and crystalline phases, similar to the transitions between the gas, liquid and solid phases of atoms and small molecules. However, the transitions in protein solutions also show many distinct features. Most notably, coexisting dilute and concentrated liquid phases in protein solutions are metastable with respect to crystallization. Furthermore, phenomena such as amorphous aggregation or regular self-assembly, which are common in protein solutions, rarely are exhibited by small molecules. We argue that all these unique features of protein phase behavior stem from the short-range and highly anisotropic nature of the protein interactions and formulate a simple model that provides a correct description of phase diagram of globular protein solutions.

We present experimental results for liquid-liquid phase separation, crystallization and aggregation of several gamma crystallins, globular proteins from eye lens involved in cataract disease. In particular, we compare the phase diagrams of several mutant human gamma-D crystallins that differ by a single point mutation. Remarkably, the solubility of these mutants decreases with increasing temperature in sharp contrast to the behavior of the native protein. At the same time liquid-liquid coexistence curve remains unaffected by mutations. We show how this apparent discrepancy is easily explained in a framework of our "aeolotopic" model which takes into account the short range and anisotropy of the protein interaction. We argue that this type of models could provide adequate theoretical basis for the analysis of protein crystallization and other protein condensation phenomena.

1834-Symp Morphology of Dense Colloidal Phases

David Weitz

Harvard University, Cambridge, MA, USA.

Colloidal particles exhibit some behavior that is similar to that of proteins in terms of their phase and crystallization behavior. While colloids are not completely like proteins, they can serve as a model with which to understand some of the underlying phase behavior. This talk will review the phase behavior of colloids, both crystals and gels. This can serve as a benchmark to which the much more complex behavior of proteins can be compared.

1835-Symp Coupled Computer Simulations and Experiments of Relevance to Alzheimer Disease

H. Eugene Stanley

Boston University, Boston, MA, USA.

This talk will describe recent in silico studies of amyloid beta-protein carried out by a group of computational physicists at Boston University in strong collaboration with a group at Harvard Medical School (now at UCLA). Recently published results [1–4] will be emphasized, and all papers can be downloaded from http://polymer.bu.edu/hes/ad/

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1836-Symp Nucleation of Condensed Phases In Supersaturated Lysozyme Solutions

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Proteins in aqueous suspensions can phase separate into highly concentrated liquid or solid phases. Protein phase separation shares many similarities with the thermodynamic and kinetics of phase separation in colloidal systems. Most prominently, metastable liquid phase separation predicted for colloids is readily observed in protein solutions. Similarly, the intricate kinetics of phase separation can prevent either system from reaching its equilibrium configuration.

Using static and dynamic light scattering, we have investigated phase nucleation in supersaturated solutions of hen egg white lysozyme. Solid-phase nucleation measurements in this system are easily distorted by the presence of frequently overlooked, contaminating lysozyme clusters. These clusters dramatically enhance nucleation rates, leading to large numbers of poor quality crystals. Nucleation rates with clean stock materials indicate that bulk crystal nucleation rates are negligible compared to nucleation at solution interfaces. In contrast, protein solutions readily undergo liquid-liquid phase separation. There are several potential explanations for the disparity in nucleation rates of ordered *vs.* disordered phases under identical solution conditions. One hypothesis is that only solid-phase nucleation requires shedding of parts of the protein's hydration layer. This kinetic barrier should be sensitive to the presence of either chaotropic or cosmotropic ions that disrupt or enhance water structure. To explore this possible connection, we investigated how miscellaneous chaotropic *vs.* cosmotropic salt-ion pairs affect vaious aspects of protein- and solution dynamics. Changes in bulk water structure were evaluated by viscosity measurements, while changes in protein hydration were monitored by measuring the protein's hydrodynamic radius. Using static and dynamic light scattering, salt-specific effect on both direct and hydrodynamic protein interactions were ascertained over a wide range of salt concentrations and solution temperatures.

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Platform AR: Calcium Signaling

1837-Plat Glycosylation Of The Inositol 1,4,5-trisphosphate Receptor Alters Whole Cell Calcium Signaling

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Calcium is a ubiquitous intracellular signalling compound. The inositol 1,4,5-trisphosphate receptor (InsP3R) is an intracellular calcium channel found within the endoplasmic reticulum. Upon cell stimulation, extracellular signals are transduced through the cytosol leading to activation of the InsP3R and release of calcium from the endoplasmic reticulum. We previously found that the InsP3R is posttranslationally modified by the addition of O-linked β-N-acetylglucosamine (O-GlcNAc), and that this modification reduces the single channel open probability. It was unclear whether this alteration in single channel function would correlate to a change in the whole cell calcium response to an InsP3-generating agonist. To address this question, we examined the effect of O-GlcNAc on InsP3-dependent calcium signalling in SH-SY5Y cells, a cultured human neuroblastoma cell line. We first show that treatment for 72 h with 8 mM extracellular GlcNAc is sufficient to increase the O-GlcNAcylation state of the InsP3R. Using Fluo-4 AM, a calcium sensitive cell-permeant fluorophore, we show that after sugar treatment fewer cells generate a calcium signal in response to 50 nM ATP stimulation. However, all cells can be induced to generate a calcium response given sufficient stimulation. Of the cells that do respond to a higher stimulus (1 μM ATP), the peak amplitude of the calcium signal is reduced in GlcNAc-treated cells. To ensure that the response is due to an effect at the level of the InsP3R, we loaded cerebellar interneurons with Oregon green BAPTA, a green fluorescent calcium indicator, and caged InsP3. We show that cells loaded with UDP-GlcNAc have a dramatically reduced calcium response to photorelease of InsP3. These results show that O-GlcNAcylation is an important regulator of the $InsP_3R$ and suggest a mechanism for altered signalling under conditions where O-GlcNAc is high, such as diabetes.

1838-Plat Intracellular Calcium Dynamics Mediated By NMDA Receptors In Retinal Horizontal Cells

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